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14. ABSTRACT The goal of this project is to evaluate the hypothesis that traumatic brain injury induces alterations in the brain's reward circuitry which may make an injured brain more susceptible to the rewarding effects of opioids. We are currently conducting experiments to evaluate the hypothesis that TBI causes changes in the analgesic response to opioids following acute and repeated drug administration. We are also testing the hypothesis that moderate TBI increases the susceptibility for opioid abuse as measured by an alteration in the rewarding properties of oxycodone. We have completed the third year of experimentation and thus far have found that TBI induces changes in oxycodone abuse-related behaviors and may induce alteration in the brain reward, particularly expression of the dopamine receptor subtype 2. All studies are on-going.

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Introduction:

Progress Report for DoD Peer Reviewed Medical Research Program of the Office of the Congressionally Directed Medical Research Program FY10 Investigator-Initiated Research Award: Partnering PI Option Application entitled "Opioid Abuse after TBI"

This report was prepared by Candace L. Floyd, Ph.D. for the work conducted in collaboration with Katherine L. Nicholson, DVM, Ph.D.

Our progress in completion of the aims is detailed below. This report focuses on the work conducted at UAB. However, it is important in emphasize that Dr. Floyd and her staff traveled to VCU to induce the traumatic brain injury (TBI) in all animals and the details of the behavioral studies are included in the report from the partnering PI, Dr. Nicholson. A brief description of the aims, the work conducted, and the data collected respective to the statement of work is provided after the original text of the aims and statement of work, which are denoted by bolded and italicized font, respectively.

Aim 1: Evaluate the hypothesis that moderate TBI causes changes in the analgesic response to opioids following acute and repeated drug administration.

Aim 2: Investigate the hypothesis that moderate TBI increases the susceptibility for opioid abuse as measured by an alteration in the rewarding properties of oxycodone.

Aim 3: Evaluate the propensity for development of physical dependence to opioids following moderate TBI.

Body:

A. Progress on Year 3 Tasks completed by UAB personnel (Dr. Floyd):

- *Task 1: Travel to VCU to induce lateral fluid percussion TBI in rats in months 1, 3, 5, 7 and 9 of year 3, as described above*

Dr. Floyd conducted all the TBI procedures in year three such that nearly all the animals need to meet the production goals were collected. As a few of the animals still need to be completed, please see the table in the VCU report, Drs. Floyd and Nicholson requested a 1 year no cost extension to complete the few remaining animals. This will likely require 1-2 more trips for Dr. Floyd to VCU to induce the TBI.

Briefly as we have previously described[1, 2], traumatic brain injury was induced in adult, male Sprague Dawley rats by lateral fluid percussion. Uninjured controls (sham group) received all surgical procedures with the omission of the fluid percussion impact, as we have previously described. Data from some of these animals is shown below and behavioral data components from all animals in the study are listed in Dr. Nicholson's report.

- *Task 2: Continue histological and biochemical analysis of cell death/ gliosis, DA signaling, opioid receptor expression and growth factors from rodent brains received from VCU*

This task in on-going and experiments and results are continuing to be collected in support of this task. An update on the preliminary data is listed below.

For these data, a 2 mm tissue punch was used to extract the brain regions of interest which included the ventral tegmental area (VTA), amygdala (AMG), cortex (CTX), nucleus accumbens (NAC), and hippocampus (HIP). Tissue samples were homogenized and then immunoblotting was conducted to assess expression of the mu opioid receptor, dopamine receptor subtype 1, dopamine receptor subtype 2, dopamine transporter, and tyrosine hydroxylase. Twenty-five micrograms of protein was used for all immunoblots and all blots were incubated in the primary antibody overnight at 4°C and blocked for 1 hour in 5% bovine serum albumin at room temperature. The immunoblots were incubated in secondary antibodies for 1 hour at room temperature. The following antibodies were used:

Primary Antibodies:

Mu Opioid receptor: Alomone Labs (catalog #AOR-001) 1:200 dilution in 1% BSA

D1 receptor: Alomone Labs (catalog #ADR-001) 1:200 dilution in 1% BSA

D2 receptor: Alomone Labs (catalog #ADR-002) 1:200 dilution in 1% BSA

Dopamine transporter: Alomone Labs (catalog #AMT-003) 1:200 dilution in 1% BSA

Tyrosine Hyroxylase: Life Tech (catalog #36990) 1:1000 dilution in 1% BSA

β-Actin: Cell Signaling (catalog #3700P) 1:4000 dilution in 1% BSA

Secondary Antibodies:

(For all blots except actin) Goat anti-rabbit: Biorad : (catalog #170-6515) 1:2000 dilution in 1% BSA

(For actin blots only) Goat anti-mouse: Biorad : (catalog #170-6516) 1:4000 dilution in 1% BSA

Immunoblots were annalyzed in the liner range by realative optical density of each band. All comparison groups were evaluted on the same blot and optical density values were normalized to β-actin as a loading control. Normalized optical density values were averaged for comparisons across immunoblots.

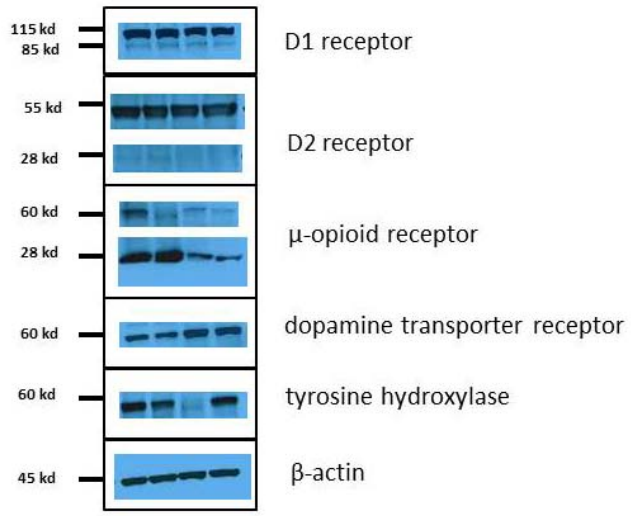

Figure 1: Representative immunoblots from regions of rodent brains taken from subjects who received moderate lateral fluid percussion TBI (two right lanes) or uninjured control (two left lanes). D1= dopamine receptor subtype 1, D2= dopamine receptor subtype 2.

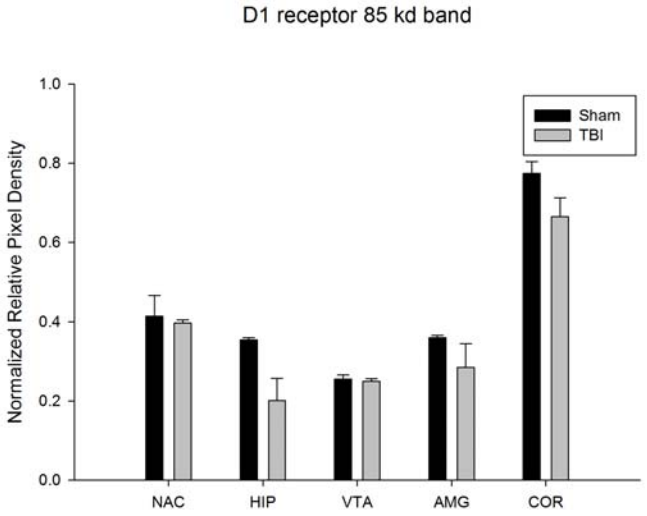

Figure 2: Quantification of the dopamine 1 receptor subtype at the 85kd molecular weight comparing the uninjured control (sham) to TBI groups at 5 days post-injury. The ipsilateral side was evaluated in the following brain regions: nucleus accumbens (NAC), hippocampus (HIP), ventral tegmental areas (VTA), amygdala (AMG), and cortex (COR).

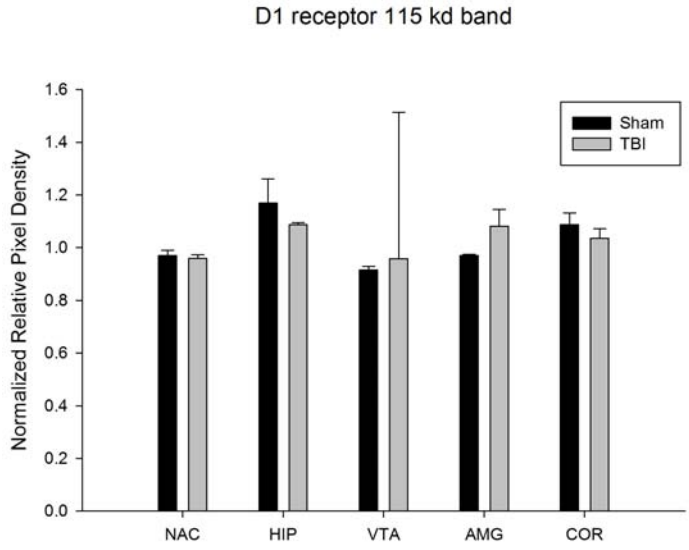

Figure 3: Quantification of the dopamine 1 receptor subtype at the 115 kd molecular weight comparing the uninjured control (sham) to TBI groups at 5 days post-injury. The ipsilateral side was evaluated in the following brain regions: nucleus accumbens (NAC), hippocampus (HIP), ventral tegmental areas (VTA), amygdala (AMG), and cortex (COR).

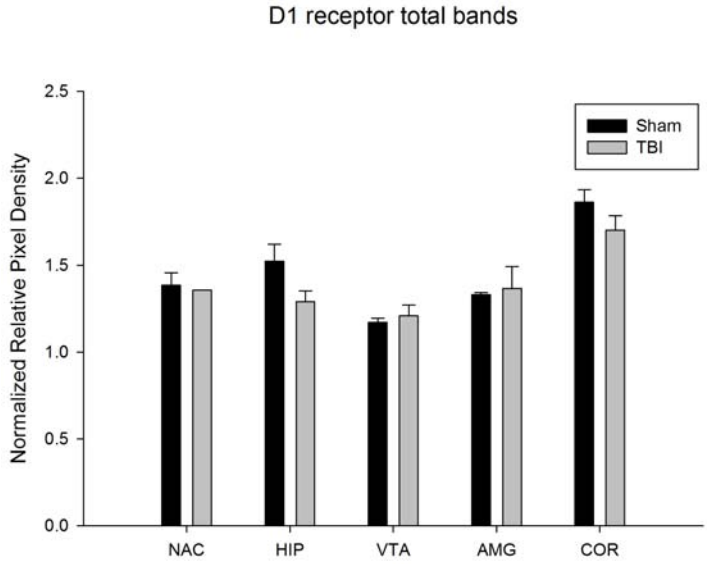

Figure 4: Quantification of the dopamine 1 receptor subtype averaged from the 85 kd and 115 kd molecular weight comparing the uninjured control (sham) to TBI groups at 5 days post-injury. The ipsilateral side was evaluated in the following brain regions: nucleus accumbens (NAC), hippocampus (HIP), ventral tegmental areas (VTA), amygdala (AMG), and cortex (COR).

With regard to the dopamine receptor subtype 1 at 5 days post-TBI, we found modest trends toward reduction of expression in the brains of subjects that received TBI as compared to uninjured controls, particularly in the hippocampus and cortex. Also, we observed a trend toward increased expression of DA1 In the amygdala and large variability in the VTA. However after analysis of variance, none of these trends reached a level of statistical significance. Taken together, these data suggest that at 5 days post-TBI, there are not robust changes in the expression of the dopamine receptor 1 subtype.

Next, we evaluated the effect of TBI on expression of the dopamine receptor 2 subtype from the same brain samples as described above.

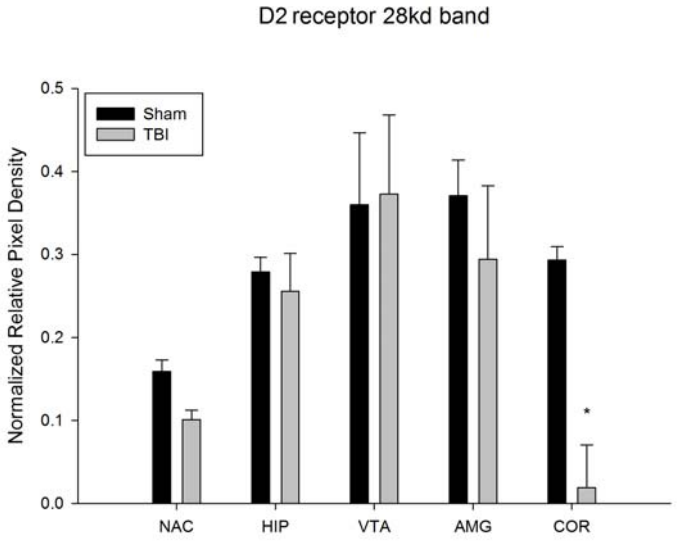

Figure 5: Quantification of the dopamine 2 receptor subtype at the 28kd molecular weight comparing the uninjured control (sham) to TBI groups at 5 days post-injury. The ipsilateral side was evaluated in the following brain regions: nucleus accumbens (NAC), hippocampus (HIP), ventral tegmental areas (VTA), amygdala (AMG), and cortex (COR). *=$p<0.05$.

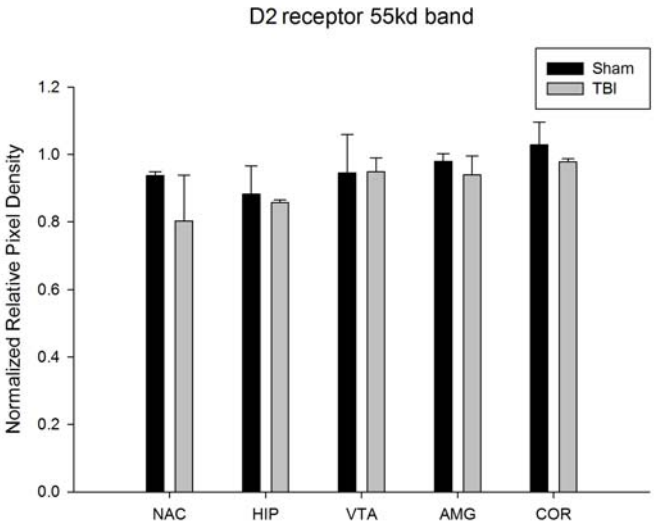

Figure 6: Quantification of the dopamine 2 receptor subtype at the 55kd molecular weight comparing the uninjured control (sham) to TBI groups at 5 days post-injury. The ipsilateral side was evaluated in the following brain regions: nucleus accumbens (NAC), hippocampus (HIP), ventral tegmental areas (VTA), amygdala (AMG), and cortex (COR).

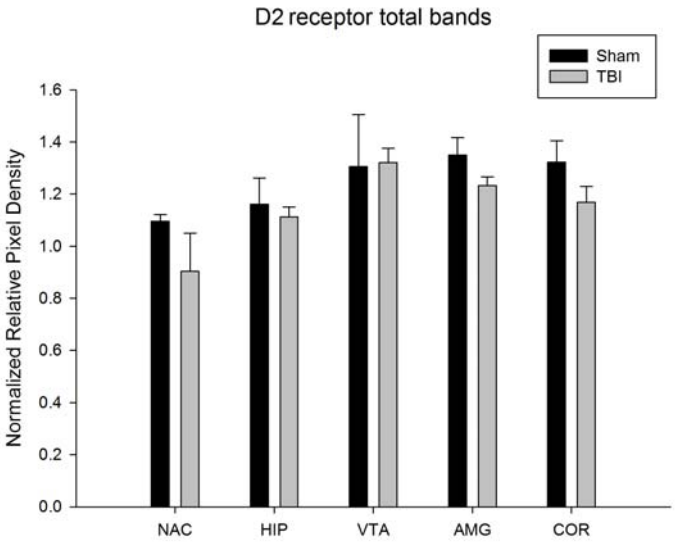

Figure 7: Quantification of the dopamine 2 receptor subtype averaged from the 28kd and 55kd molecular weight comparing the uninjured control (sham) to TBI groups at 5 days post-injury. The ipsilateral side was evaluated in the following brain regions: nucleus accumbens (NAC), hippocampus (HIP), ventral tegmental areas (VTA), amygdala (AMG), and cortex (COR).

With regard to the dopamine receptor subtype 2, analysis of the band at the 28kd molecular weight revealed robust decreases in the expression nucleus accumbens and cortex in subjects that received TBI. This reached statistical significance in the cortex. However, analysis of the 55kd molecular weight band the reduction in expression of D2 in the injured brains was much less robust. When densitometry of both molecular bands was averaged, no robust differences in the expression of the D2 receptor were observed, likely due to the influence of the 55kd band values. Thus, when taken together, robust decreases in the D2 receptor subtype were observed in the cortex (statistically significant) and nucleus accumbens when only the 28 kd molecular weight band was considered.

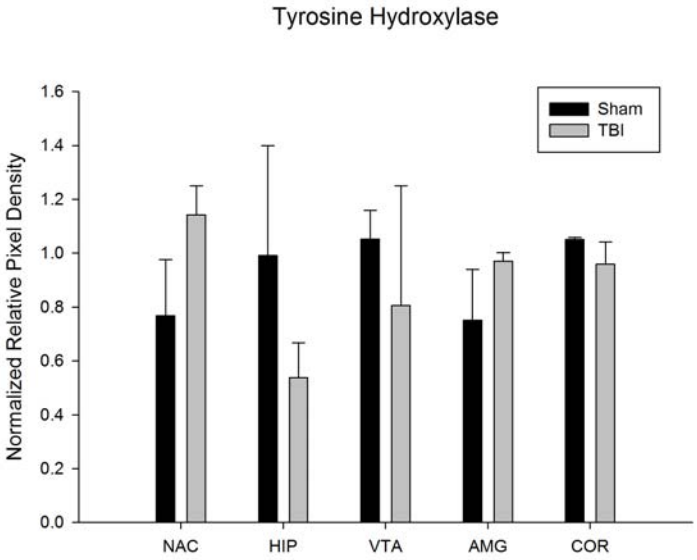

Figure 8: Quantification of the expression of tyrosine hydroxylase levels comparing the uninjured control (sham) to TBI groups at 5 days post-injury. The ipsilateral side was evaluated in the following brain regions: nucleus accumbens (NAC), hippocampus (HIP), ventral tegmental areas (VTA), amygdala (AMG), and cortex (COR).

Next, we evaluated the effects of TBI on the expression of tyrosine hydroxylase (TH), the enzyme responsible for catalyzing the conversion of tyrosine to L-DOPA and a key component in dopamine synthesis. Interestingly, the trends in TH expression varied across the brain region. We observed a trend to increased TH in the nucleus accumbens and reduced TH in the hippocampus in the TBI group as compared to the uninjured control. Other brain regions were either more variable in the expression of TH after TBI (i.e. VTA) or we did not observe a robust difference in TH expression after TBI (i.e. amygdala and cortex). Taken together, these data suggest that TBI induces increases in dopamine synthesis in the nucleus accumbens and decreases in the hippocampus, however these trends did not reach statistical significance and more evaluation is needed.

Our next evaluation was the expression of the mu opioid receptor after TBI across these brain regions.

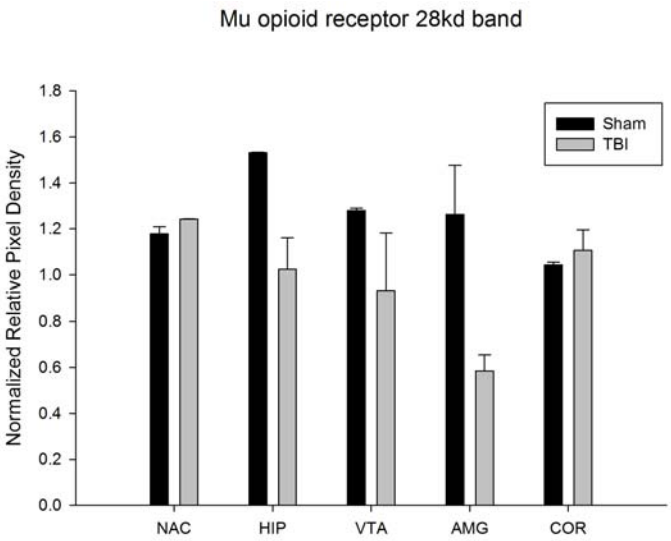

Figure 9: Quantification of the mu opioid receptor at the 28kd molecular weight comparing the uninjured control (sham) to TBI groups at 5 days post-injury. The ipsilateral side was evaluated in the following brain regions: nucleus accumbens (NAC), hippocampus (HIP), ventral tegmental areas (VTA), amygdala (AMG), and cortex (COR).

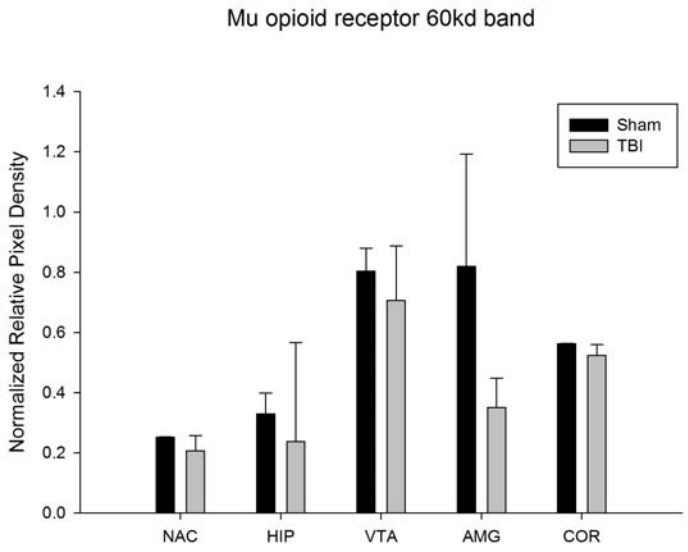

Figure 10: Quantification of the mu opioid receptor at the 60kd molecular weight comparing the uninjured control (sham) to TBI groups at 5 days post-injury. The ipsilateral side was evaluated in the following brain regions: nucleus accumbens (NAC), hippocampus (HIP), ventral tegmental areas (VTA), amygdala (AMG), and cortex (COR).

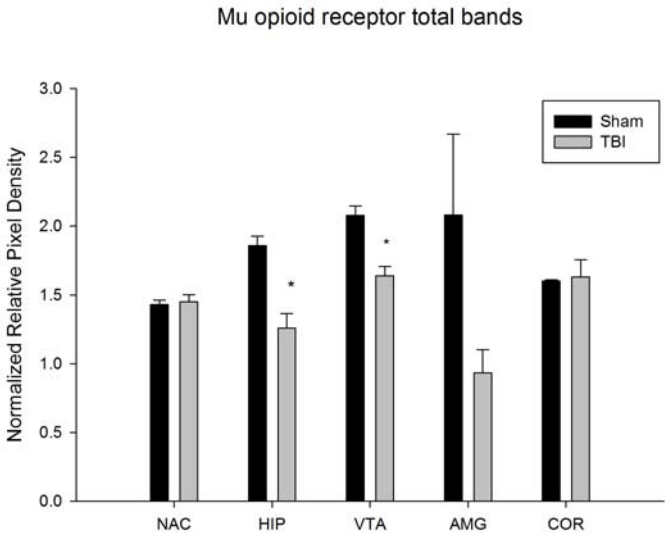

Figure 11: Quantification of the mu opioid receptor subtype averaged from the 28kd and 60kd molecular weight comparing the uninjured control (sham) to TBI groups at 5 days post-injury. The ipsilateral side was evaluated in the following brain regions: nucleus accumbens (NAC), hippocampus (HIP), ventral tegmental areas (VTA), amygdala (AMG), and cortex (COR). *=$p<0.05$.

With regard to the expression of the mu opioid receptor after TBI, we found that there were trends for reduction in expression after TBI in the hippocampus, VTA, and amygdala when assessing the 28kd molecular weight. However, these trends did not reach statistical significance. Similar trends were observed for analysis of the 60kd molecular weight band in that TBI reduced expression of the mu opioid receptor in the hippocampus, VTA, and amygdala. These trends did not reach statistical significance. When the data from the 28kd band and the 60kd band were averaged, a statistically significant reduction in expression of the mu opioid receptor was observed in the hippocampus and VTA. Also, a strong non-significant trend for TBI-induced reduction in the mu opioid receptor expression was observed in the amygdala. Taken together, these data suggest that TBI induces a reduction in the expression of the mu opioid receptor in key brain regions associated with reward circuitry, including that hippocampus, VTA and amygdala. Subsequent work is need to further evaluate these trends.

- *Task 3: Lead preparation of peer-reviewed manuscript(s) to report results.*

This task is also on-going. We are in the middle stages of preparation of the first manuscript and expect to have that submitted by the end of 2014. We then plan to submit the second manuscript in early 2015.

Key Research Accomplishments:

- **Taken together, these data indicate that TBI may alter brain reward circuitry, particularly dopaminergic signaling and expression of the mu opioid receptor.**

Reportable Outcomes:

- Presentation of data by Dr. Floyd in the Associated in Emergency Medical Education and Alliance for Global Narcotics Training Conference at the Defense Health Headquarters in March 2014
- Submission of abstract for presentation of data to Military Health System Research Symposium in 2014 (abstract not accepted for presentation)

Conclusion:

The data for the project (combining both VCU and UAB efforts) thus far suggest that moderate/severe traumatic brain injury induces a change in the response to oxycodone such that injured subjects are more likely to abuse oxycodone and less sensitive to the negative effects the drug. This is likely due to changes in the brain reward circuitry induced by injury, particularly with dopaminergic signaling and expression of mu opioid receptor.

Reference List

(1) Day NL, Floyd CL, D'Alessandro TL, Hubbard WJ, Chaudry IH. 17beta-estradiol confers protection following traumatic brain injury in the rat and involves activation of G Protein-coupled estrogen receptor 1 (GPER). *J Neurotrauma* 2013.

(2) Floyd CL, Golden KM, Black RT, Hamm RJ, Lyeth BG. Craniectomy position affects morris water maze performance and hippocampal cell loss after parasagittal fluid percussion. *J Neurotrauma* 2002;19(3):303-316.